Plant responses to water stress
Role of reactive oxygen species

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Responses of plants to water stress may be assigned as either injurious change or tolerance index. One of the primary and cardinal changes in response to drought stress is the generation of reactive oxygen species (ROS), which is being considered as the cause of cellular damage. However, recently a signaling role of such ROS in triggering the ROS scavenging system that may confer protection or tolerance against stress is emerging. Such scavenging system consists of antioxidant enzymes like SOD, catalase and peroxidases, and antioxidant compounds like ascorbate, reduced glutathione; a balance between ROS generation and scavenging ultimately determines the oxidative load. As revealed in case of defense against pathogen, signaling via ROS is initiated by NADPH oxidase-catalyzed superoxide generation in the apoplastic space (cell wall) followed by conversion to hydrogen peroxide by the activity of cell wall-localized SOD. Wall peroxidase may also play role in ROS generation for signaling. Hydrogen peroxide may use Ca^{2+} and MAPK pathway as downstream signaling cascade. Plant hormones associated with stress responses like ABA and ethylene play their role possibly via a cross talk with ROS toward stress tolerance, thus projecting a dual role of ROS under drought stress.

Introduction

Earth surface that constitutes the Nature has become suitable to sustain life through changes in a long course of time. This suitability is based on average conditions having otherwise extremes in respect of environmental factors creating marginal habitats. Plants and animals living in such habitats are being challenged by harsh environment and consequently they have evolved adaptive strategies to survive. Accordingly, such environmental profile determines the natural distribution of plant species. Again, gradual changes in environment with time call for a change in successive vegetation pattern differing in adaptive strategies. Besides such spontaneous changes that occur through ages plants are now subjected to threats of climate changes emerging from global warming that has been a major issue to all the nations for

last few decades. This has been a great concern for biodiversity that is being lost at faster rate.

Water Stress and Plant Responses

Water stress or drought is one of the consequences of erratic rainfall and becomes a constraint to the plants, both wild species as well as crop plants. Moreover, most soil has a variable wettability resulting in a heterogeneous moisture profile following precipitation or irrigation.[1] Plants naturally distributed in dry habitats with water scarcity are armoured with constitutive morphological and anatomical modifications that help to conserve water. This kind of resistance is usually referred to as drought avoidance or postponement. On the other hand, mesophytes having different degree of tolerance to water stress mostly rely upon metabolic adaptations. Mechanism of such tolerance entails a variation in the detail network and cascade of events or reactions leading finally to alleviation of potential stress-induced cellular injuries depending on the plant species that have evolved through environmental changes.

Like all other stresses, water stress may also be imposed gradually or suddenly in nature as well as in agricultural conditions, though a gradual decline in soil water content is more common under field conditions. Depending on the speed of imposition and magnitude of stress, responses of plants to stress vary. Levitt[2] has compiled the responses of plants to stresses as injuries and adaptive changes that provide a coherent frame of reference. Water stress, in particular, is an environmental constraint often faced by plants in their life cycles limiting survival, reproduction and yield. Plant responses to water stress or drought have been critically reviewed by a number of authors.[3-7] Some of these responses have adaptive value in conferring protection while others might represent cellular damage caused by water deficit.[8,9] Drought stress often exacerbates the effect of other stresses and, at the same time, several different abiotic stresses may result in water stress.[10]

Responses of plants to stress may be revealed at a whole plant level as an integrated tissue system while some of the responses occur at the cellular level. However, in case of the former also some changes in the cellular level are to be considered pivotal for the final response but requiring the presence or activities of cells of other parts of the plant. For example, closing of stomata results from some subtle biochemical and molecular changes in the guard cell itself but this event is ultimately induced by the

signaling transduced by other cells like root cells. Sometimes a comparison between cellular response and whole plant response may reveal the level of organization where the adaptation operates.[11] Besides, physiological stage for studying stress responses also becomes important. Thus seeds are very unique system that behaves differently.[12] Seed germination and seedling growth declines with increasing water stress[13,14] while proteolysis in cotyledons during storage mobilization is retarded by water stress.[15] Metabolic changes that occur in plants, particularly mesophytes, in response to water stress have been major targets while searching for molecular mechanism of stress tolerance.[6] Such studies have been strengthened further with the aid of molecular tools like microarray and differential expression of genes.[16]

Reactive Oxygen Species (ROS) and Oxidative Metabolism

It is now well established that virtually all biotic and abiotic stresses induce or involve oxidative stress to some degree, and the ability of plants to control oxidant levels is highly correlated with stress tolerance.[17] Drought stress is reported to increase the production of reactive oxygen species (ROS) and increase the oxidative load in plants.[18] An increase in ROS levels can provoke a partial or severe oxidation of cellular components inducing redox status changes, so continuous control of ROS and therefore of their metabolism is decisive under stress conditions.[19] Metabolism of ROS is of paramount importance in relation to control of oxidative physiology of plants. The kinds of ROS that have been investigated in plants include hydrogen peroxide (H_2O_2), superoxide anion ($O_2 \cdot^-$), hydroxyl radicals (.OH), singlet oxygen ($^1O_2$) and nitric oxide (NO). H_2O_2, $O_2 \cdot^-$ and $\cdot OH$ can convert to one another.[20,21] Such conversion may occur spontaneously or catalyzed by enzymes.[22] ROS metabolism in plants has become a frontier of research considering the differential action of ROS depending on the cellular compartments of their occurrence forming a complex network.[23]

In the ROS family hydrogen peroxide assumes most importance as regard to its various roles in plant growth, development and metabolism. It is not a free radical, but potentially reactive oxygen that becomes more damaging in presence of transition metal (e.g., Fe^{2+}). Extensive studies have demonstrated the role of hydrogen peroxide during stress and senescence as deleterious to the cellular macromolecules as well as regulators in case of different plant processes.[17,21,24-26] Compared with hydrogen peroxide superoxide and hydroxyl radicals are less stable and cannot cross the membrane that makes them less suitable as signal molecule. Under normal conditions, the half-life of H_2O_2 is probably 1 ms, while that of superoxide and singlet oxygen is at the level of microseconds and hydroxyl radical is quite unstable (1 ns).[27] However, these latter forms are more reactive than H_2O_2 causing severe damage locally.

Balance between ROS Production and ROS Scavenging

Site and physiological stage of ROS generation play important role in specific action of ROS.[19] In green plant parts major source

of ROS is the chloroplast where the photosynthetic electron transport system may become overactive causing a spillover of reducing power that is responsible for reduction of oxygen to different ROS.[28,29] Though such ROS generation occurs under normal condition, it is much more aggravated under stresses.[30] Similar enhanced production of ROS, particularly H_2O_2, also occurs in leaves during senescence in light causing chlorophyll degradation, which can be retarded by treatment with DCMU, an electron transport inhibitor.[24] Peroxisomes are another site of superoxide and H_2O_2 production because of several key metabolic reactions including photorespiration[31] and are the major contributor to the cellular pool of H_2O_2.[18] On the other hand, mitochondrial electron transport is also a source of ROS like superoxide and H_2O_2 particularly under stresses.[32] ROS are also generated at the plasma membrane level or extracellularly in the apoplast though a transmembrane enzyme, NAD(P)H oxidase, which transfers electrons from cytoplasmic NAD(P)H to O_2 to form $O_2 \cdot^-$ and subsequently H_2O_2 and $OH \cdot$.[33,34] In addition to NADPH oxidase, pH-dependent peroxidase, germin-like oxalate oxidases and amine oxidase have also been proposed to be the source of H_2O_2 in the apoplast.[35]

The evolution of aerobic metabolism unavoidably brought the plants under the threat of ROS that are produced both under normal and stressed condition, the balance being tilted in the latter. Accordingly, plants have evolved the protection or defense mechanism to scavenge ROS by antioxidant system, both enzymatic and non-enzymatic. Among the antioxidant enzymes superoxide dismutases (SODs) that can dismutate $O_2 \cdot^-$ into more stable H_2O_2, constitute the first line of defense against ROS and are present in all subcellular locations.[36] Based on the requirement of metal cofactor SODs are classified into three groups: Fe SOD, Mn SOD and Cu-Zn SOD. Fe SODs are located in the chloroplasts, Mn SODs are in mitochondria and peroxisomes and Cu-Zn SODs in the chloroplasts, the cytosol and the extracellular (apoplast) space.[36] Hydrogen peroxide is subsequently detoxified by enzymes like catalase, ascorbate peroxidase and glutathione peroxidase. Catalase can act on H_2O_2 directly, while the latter enzymes require ascorbate and glutathione, respectively, as electron donors.[30] Catalase that has low affinity for H_2O_2 thereby removing the bulk of H_2O_2, is localized mostly in peroxisomes. On the other hand, ascorbate peroxidase has a higher affinity for H_2O_2 and is found in chloroplasts, mitochondria, peroxisomes and cytsol. Thus ascorbate peroxidase being located in every ROS producing compartment may function as a fine regulator of intracellular ROS level.[10]

Among the nonenzymatic antioxidants the major cellular redox buffers are ascorbate and glutathione (GSH), both of which are present in different cellular compartments. Mutants with decreased ascorbic acid levels or altered GSH content are hypersensitive to stress. GSH is oxidized by ROS forming oxidized glutathione (GSSG) and ascorbate is oxidized to monodehydroascorbate (MDA) and dehydroascorbate (DHA), which can be reduced back to GSH and ascorbate, respectively through the ascorbate-glutathione cycle[37] that plays important role in ameliorating oxidative stress. Other antioxidant compounds playing important role in ROS scavenging are tocopherol, flavonoids, alkaloids and carotenoids.

An appropriate intracellular balance between ROS generation and scavenging is maintained as a result of efficient coordination of reactions in different cell compartments and is governed by a complex network of prooxidant and antioxidant systems.[21] Such balance may be perturbed by stresses including drought; however, based on the capacity to maintain or re-establish the redox homeostasis a plant species may be said as tolerant to stress. Thus, besides the levels of osmolytes like proline and sugars,[38] comparative changes in activities of antioxidant enzymes in response to water stress have often been used for selection of drought resistant plant.[39,40] Again, preferential scavenging of particular ROS occurs depending on the cellular compartment, physiological stage and plant species resulting in differential ROS signature of the stressed cell or plant. Thus both a decline and increase in catalase and SOD activity has been recorded under water stress, although transgenic plants overexpressing these enzymes acquire higher tolerance to drought stress as well as oxidative stress than respective wild plants.[10] Differential sensitivity and affinity of catalase and ascorbate peroxidase to ROS is suggestive for their differential role in ROS scavenging—catalase counteracts excessive production under severe drought stress and reducing the ROS level that is scavenged subsequently by APX and ascorbate-glutathione cycle.

Dual Action of ROS: Damaging and Signaling for Protection

A steady-state cellular ROS level prevails under normal conditions because of a balance between ROS generation and scavenging. Stresses including drought stress shift this balance with more ROS being produced than are metabolized creating an oxidative stress. But before reaching this point when ROS production overwhelms scavenging action an early rise in ROS level can act as a signaling for acclimation or defense response. Such a signaling role of ROS has been understood well in case of defense against pathogens where an oxidative burst initiates the process of signaling for defense.[41] Indeed ROS have now been recognized to serve positive roles in growth and development apart from their destructive role induced by stresses. Thus ROS including H_2O_2 have been demonstrated to play role in radicle emergence during seed germination,[42,43] root growth and root hair elongation[44,45] as well as defense against pathogens during seed germination.[46]

Relative stability of H_2O_2 compared with other ROS and its ability to cross the membrane makes it suitable for signaling. It can induce the intracellular ROS scavenging system by activating the antioxidant enzymes and also by modulating the expression of genes of these enzymes.[47] For the purpose of signaling, either in case of stress responses or growth and development, the site and amount of ROS production should be under tight control. One of the most important sites to initiate the defense or acclimation response via ROS must be the cell wall. Most vulnerable enzyme for ROS production in this compartment is NADPH oxidase that produces $O_2^{\cdot-}$, which, in turn, is dismutated to H_2O_2 either spontaneously or by the action of cell wall localized SOD.[45] Wall-bound peroxidases, apart from acting as a H_2O_2 scavenger, also play a role in ROS signaling by generating ROS like $O_2^{\cdot-}$ and

H_2O_2, the latter may also serve as the substrate for lignin required for cell wall composition. These peroxidases are grouped under class III peroxidases that being located in cell wall play a diverse role in plants.[48,49] Their role in defense against pathogens is well defined.[50] The specificity of the cellular ROS signal can be determined by its site of production, control and transduction.[29] Hence the different plant cell compartments will influence differentially the setting of the cellular redox signal under drought stress.

Downstream signaling of ROS or hydrogen peroxide is likely to occur via calcium and reversible protein phosphorylation.[25,26] Changes in cytosolic free calcium ($[Ca^{2+}]_{cyt}$) have often been reported under abiotic and biotic stresses and ROS including H_2O_2 can activate hyperpolarization-activated calcium channels (HACCs) present on the plasma membranes[51] to increase the Ca^{2+} influx. Intracellular Ca^{2+} can again stimulate directly NADPH oxidase to produce ROS in the apoplast thus constituting a positive feedback loop.[52] On the other hand, reversible protein phosphorylation is reported to be involved in downstream signaling following ROS generation[25] and several types of protein kinases have been shown to be activated by H_2O_2,[53] but such activation is probably not mediated by Ca^{2+}, as no Ca^{2+}-dependent kinase under H_2O_2 regulation has been reported in reference 25. However, several reports are there connecting H_2O_2 with mitogen activated kinase (MAPK) signaling cascade, which, in turn, can modulate gene expression via activation of transcription factors.[10,25,26] Such regulated genes seem to be involved in cellular protection and repair process as some of the gene products are known for desiccation tolerance and DNA damage repair.[54] A cDNA microarray study in Arabidopsis has shown that H_2O_2 can upregulate 113 genes while downregulate 62 genes which suggests that H_2O_2 is likely to play a key role in orchestration of plant drought responses modulating Ca^{2+} signaling, MAPK cascades and gene expression.[55] Besides activating MAPK, H_2O_2 can inhibit protein phosphatases like PP2C by oxidizing directly cysteine residues; such PP2Cs (ABI1 and ABI2) are negative regulators of ABA action.[26] Thiol modification could be one way of H_2O_2 perception.[53] Indeed, because of small size of H_2O_2, a direct interaction with proteins through thiol modification has been taken as the means for signal propagation instead of specific recognition of H_2O_2 by a receptor protein.[22,56] Another signaling molecule, nitric oxide (NO) may also react with thiol groups of proteins many of which are also found to be the targets of H_2O_2[57] thus converging H_2O_2 and NO to a common point of interaction.

Phytohormones and their Cross Talk with ROS

Plant hormones constitute a battery of regulators involved in control of physiological and metabolic processes and also likely to mediate the responses of plants to environmental stresses. In several instances ROS are implicated as second messengers working downstream of these hormones.[58] Among the hormones ABA is the most important one playing role in signaling for tolerance against stresses including drought. Drought stress induces enhanced accumulation of ABA and triggers downstream responses that confer drought tolerance to plants. One

of the well-studied responses in plants to water stress is the stomatal closing induced by ABA. Recent studies have shown that such response is mediated by ROS, particularly H_2O_2, which is synthesized by plasma membrane bound NADPH oxidase.[59,60] This is supported by the molecular evidence showing increased expression of two homologs of *rboh* (*AtrbohD* and *AtrbohF*), genes encoding catalytic subunits of NADPH oxidase, in guard cells by ABA treatment and double mutants (*atrbohD/F*) were impaired in stomatal closing and calcium channels in response to ABA and rescued by H_2O_2 application.[61] H_2O_2 mediates stomatal closure by elevating cytosolic Ca^{2+} level through activation of channels.[60,62,63] Evidence on direct inactivation of two type 2C phosphatases, PP2Cs (ABI1 and ABI2) that function as negative regulators of ABA signaling, by H_2O_2 further strengthen the cross talk between ABA and H_2O_2 in closing of stomata induced by water stress.[64] Added to this is one serine/threonine protein kinase (*OST1*) that acts upstream of ABA-induced ROS production during stomatal closure.[65] At least in one case ROS has been shown to be involved in drought-induced ABA synthesis.[66]

Another hormone often associated with stress is ethylene, production of which is enhanced by stresses including osmotic stress and such synthesis is dependent on ROS.[67] Similar ROS-induced ethylene synthesis has also been reported in case of wound response and defense against pathogens.[68,69] Ethylene has also been implicated, at least in some plants, for stomatal closure and ETR1, one of the ethylene receptors, is involved in sensing H_2O_2 and subsequent stomatal closure.[70] Another possible point of cross talk would be MAPK cascade that may be shared by both H_2O_2 and ethylene,[71] though an ethylene-independent MAPK acitivity is reported to be induced by H_2O_2.[72] A role of MAPK in case of ABA-induced stomatal closure has also been reported in reference 73. It appears that hormones either transduce signals through ROS or ROS may have a cross talk with the signaling cascades used by hormones in controlling stress responses as well as other physiological and developmental processes.

Final Words

Undoubtedly, drought stress induces ROS generation as a primary response of plant and this may be mediated by hormones like ABA and ethylene as well, which may sometimes play a downstream role too. Gross level of ROS could exacerbate the stress induced damages to most of the cellular components unless compromised because of ROS scavenging by antioxidant system. However, depending on spatial and temporal ROS generation and scavenging (ROS management) responses can be characterized as toward conferring protection by arousing the protection system or as directly leading to injuries or death. Gradual imposition of drought stress, which is more common in nature, probably triggers ROS generation in the apoplast by plasma membrane-localized NADPH oxidase where Ca^{2+} plays a role as an upstream as well as downstream messenger forming a positive feedback loop. Apoplast is ideal site for initiation for signaling by ROS (localized oxidative burst) as this compartment has less redox buffering capacity,[18] which could have otherwise diffused the signal strength. Efficiency for such an apoplast based ROS signaling system has already been appreciated in case of defense against pathogen (biotic stress). Now the fate of the cellular system subjected to water stress depends on whether intracellular ROS scavenging system, being stimulated by the ROS signal of extracellular origin, keeps the oxidative load low on the cellular components or a rapid intracellular ROS accumulation has taken over before defense system is put in place. This again depends on speed and magnitude of stress imposition as well as the plant system subject to the stress, the latter being variable genetically as regard to the chronology and propensity of ROS management toward orchestration of defense related changes under stress.

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